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The NoCut Pathway Links Completion of Cytokinesis to Spindle Midzone Function to Prevent Chromosome Breakage

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SUMMARY

During anaphase, spindle elongation pulls sister chromatids apart until each pair is fully separated. In turn, cytokinesis cleaves the cell between the separated chromosomes. What ensures that cytokinesis proceeds only after that all chromosome arms are pulled out of the cleavage plane was unknown. Here, we show that a signaling pathway, which we call NoCut, delays the completion of cytokinesis in cells with spindle-midzone defects. NoCut depends on the Aurora kinase Ipl1 and the anillin-related proteins Boi1 and Boi2, which localize to the site of cleavage in an Ipl1-dependent manner and act as abscission inhibitors. Inactivation of NoCut leads to premature abscission and chromosome breakage by the cytokinetic machinery and is lethal in cells with spindle-elongation defects. We propose that NoCut monitors clearance of chromatin from the midzone to ensure that cytokinesis completes only after all chromosomes have migrated to the poles.

INTRODUCTION

Mitosis is a highly coordinated process in which the two copies of each chromosome (sister chromatids) are segregated away from each other to opposite poles of the cell. Subsequently, the cell is cleaved between the two newly formed nuclei, leading to two independent cells. To ensure that each daughter cell inherits a single and complete copy of the genome, chromosome segregation and cell division are tightly coordinated. The fidelity of these events is ensured by complex surveillance mechanisms that detect and correct errors (for review, see Hartwell and Weinert [1989]).

The spindle checkpoint, together with kinetochore proteins and the kinase Aurora B (Ipl1 in yeast), ensures that chromosome segregation begins only after each pair of sister chromatids achieves bipolar attachment to the mitotic spindle (Musacchio and Hardwick, 2002). Chromosome segregation is then fulfilled by elongation of the anaphase spindle. Elongation is supported by the spindle midzone, which bundles antiparallel nonkinetochore spindle microtubules (Glotzer, 2005; McCollum, 2004). Numerous molecules localize to the spindle midzone. The microtubule-bundling protein PRC1 (Ase1 in budding yeast) is required for spindle stability. In addition, a number of yeast kinetochore components such as Ndc10, Ndc80, and Slk19 and the chromosomal passenger proteins Aurora B, INCENP, and survivin (Ipl1, Sli15, and Bir1 in budding yeast) also localize to the midzone (Adams et al., 2001; Bouck and Bloom, 2005; Buvelot et al., 2003; Glotzer, 2005; Sullivan et al., 2001). When the spindle becomes longer than twice the longest chromosome arm, chromosome segregation is achieved.

Cytokinesis, the final step of cell division, physically dissociates the two daughter cells from each other (Glotzer, 2005; Guertin et al., 2002). In animal cells, cytokinesis starts with contraction of the equatorial actomyosin ring, leading to membrane furrowing. As furrow ingression completes, the cells remain linked by a cytoplasmic bridge containing the remnant of the spindle midzone, the midbody. The actomyosin ring then disassembles while resolution of the plasma membrane, called abscission, completes cytokinesis.

In animal cells, spindle-midzone defects generally cause the furrow to regress, leading to the formation of binucleated cells (Glotzer, 2005; Guertin et al., 2002; McCollum, 2004). Cases of furrow regression have also been described in cells with incomplete DNA segregation (Meraldi et al., 2004a; Mullins and Biesele, 1977). However, it is not clear how and why defects in DNA segregation lead to cytokinesis failure.

Like in animal cells, yeast cytokinesis is a multistep process that also starts with the assembly and contraction of

an actomyosin ring. Contraction depends on activation of the mitotic exit network (MEN) and Cdk1 inactivation (for review, see Tolliday et al. [2001]). After contraction, the ring disassembles and the narrow cytoplasmic bridge left between the two daughter cells is resolved by abscission (Dobbelaere and Barral, 2004). Three independent molecular pathways fulfill these events. Type II myosin, Myo1, ensures actomyosin ring contraction. Hof1, homologous to *Schizosaccharomyces pombe* Cdc15p, defines a second pathway required for proper actin organization and septation. The third and least understood pathway depends on the protein Cyk3 and acts downstream of actomyosin ring contraction in septation and perhaps abscission. Inactivation of either of the *MYO1*, *HOF1*, or *CYK3* genes affects cytokinesis only mildly, whereas simultaneous disruption of any two of them abolishes cytokinesis and is lethal (Korinek et al., 2000; Tolliday et al., 2001).

Here, we investigate how cytokinesis is coordinated with anaphase. We show that similar to animal cells, yeast spindle-midzone defects prevent the completion of cytokinesis. Abscission inhibition depended on NoCut, a signaling pathway that involved the Aurora kinase, Ipl1, and the anillin-related proteins Boi1 and Boi2. Inactivation of this pathway causes chromosome breakage due to precocious abscission. Thus, our results provide a model for how the timing of cytokinesis is coordinated with chromosome segregation.

RESULTS

Ase1 Is Required for Timely Cytokinesis

In animal cells, spindle-midzone defects lead to cytokinesis failure. We therefore investigated whether the midzone is also required for cytokinesis in budding yeast. The microtubule binding protein Ase1, the homolog of human PRC1, is the only yeast protein known to localize exclusively to the spindle midzone. Yeast cells lacking Ase1 are viable and form highly unstable spindles that break down prematurely during anaphase (Schuyler et al., 2003). Tetrad analysis demonstrated that cells lacking Myo1 and Hof1 depend on Ase1 for survival, indicating that Ase1 is required for cytokinesis and suggesting that it may function in the Cyk3 pathway (data not shown). In support of this conclusion, the *ase1*Δ *cyk3*Δ double mutant was fully viable. These data indicate that Ase1 or the spindle midzone participates in yeast cytokinesis.

To characterize the role of Ase1 in cytokinesis, we visualized the plasma membrane at the bud neck of dividing *ase1*Δ cells using Ras2-GFP as a marker (Whistler and Rine, 1997). Cells that had initiated anaphase were identified by the fact that they had one spindle pole body (SPB) in the mother cell and the other in the bud. SPBs were visualized using Spc42-CFP as a marker. In ana-/telophase cells, the plasma membrane at the bud neck was in one of three states (Figure 1A): continuous ("open" neck, in which the furrow has not ingressed), contracted (the furrow had ingressed), or resolved into two (indicating that abscission was completed but mother cells had not yet separated from their buds). To determine which stage of cytokinesis was affected in *ase1*Δ mutants, we quantified the frequency of these different classes in asynchronous wild-type and *ase1*Δ cultures (Figure 1B). In wild-type, 45% of the ana- and telophase cells had not yet undergone contraction, while in 52% of the cells the membrane was clearly resolved into two. Only about 3% of the cells showed a "pinched" neck, in which the membrane had ingressed. The small fraction of cells with pinched bud necks reflects the short interval between furrow ingression and membrane resolution in wild-type cells. This category of cells was specifically and strongly increased (8-fold) in the *ase1*Δ population (Figure 1B). Thus, furrow ingression progressed properly but membrane resolution was delayed in the *ase1*Δ cells. These data are consistent with Ase1 acting in cytokinesis posterior to actomyosin ring contraction, most likely in abscission.

To further analyze the role of Ase1, we created a conditional *ASE1* shut-off allele. The Ase1 promoter was replaced by a weak version of the regulatable *GAL* promoter (GALS; Janke et al., 2004), which allowed Ase1 synthesis to be turned on to appropriate levels in galactose and turned off in glucose media. Accordingly, *GALS:HA-ASE1* cells did not contain detectable levels of HA-Ase1 protein when grown in glucose medium (Figure 1C). In these cells, the spindle failed to elongate and broke down prematurely compared to wild-type cells (see Figure 6B). Thus, the *GALS:HA-ASE1* allele allowed efficient depletion of Ase1 within a single cell cycle, consistent with Ase1 being unstable in G_1 (Juang et al., 1997). Similar to the *ase1*Δ mutant cells, the *GALS:HA-ASE1* cells delayed abscission upon shifting to the repressing medium (Figure 1B). In addition, a high fraction of *GALS:HA-ASE1* cells formed chains that failed to separate, where primary septa failed to form properly, as indicated by chitin staining (Figure 1E). These cells were not merely kept together by cell wall remnants but rather by a continuous plasma membrane, since digestion of the cell wall using zymolyase failed to dissociate the chains (Figure 1D). Together, these data indicate that Ase1 function is required for efficient resolution of the plasma membrane after furrow ingression and hence for proper abscission.

Mutations in Kinetochore Proteins that Affect the Spindle Midzone Also Prevent Normal Cytokinesis

The cytokinesis defect observed in cells lacking Ase1 suggested that the spindle midzone is involved in the control of abscission. Alternatively, cytokinesis might have been repressed until completion of chromosome segregation, which is delayed in these cells (Figure 7A). To distinguish between these possibilities, we tested whether mutations that impair chromosome segregation also impair cell cleavage. Kinetochore components fall into two classes relative to chromosome separation and spindle function. Ndc10, an inner kinetochore component, also localizes to the spindle midzone during anaphase and is required for both kinetochore assembly and midzone stability (Bouck and Bloom, 2005; Buvelot et al., 2003; see Figures S1A and S1B in the Supplemental Data available with this